



IL-10 reduces grain dust-induced airway inflammation and airway hyperreactivity

Timothy J. Quinn, Somer Taylor, Christine L. Wohlford-Lenane and David A. Schwartz

J Appl Physiol 88:173-179, 2000.

You might find this additional information useful...

This article cites 47 articles, 28 of which you can access free at:

<http://jap.physiology.org/cgi/content/full/88/1/173#BIBL>

This article has been cited by 4 other HighWire hosted articles:

Leukocyte-Derived IL-10 Reduces Subepithelial Fibrosis Associated with Chronically Inhaled Endotoxin

S. Garantziotis, D. M. Brass, J. Savov, J. W. Hollingsworth, E. McElvania-TeKippe, K. Berman, J. K. L. Walker and D. A. Schwartz

Am. J. Respir. Cell Mol. Biol., December 1, 2006; 35 (6): 662-667.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Cytokines in asthma

J.C. Kips

Eur. Respir. J., July 2, 2001; 18 (34_suppl): 24S-33s.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

LPS-induced bronchial hyperreactivity: interference by mIL-10 differs according to site of delivery

V. Deleuze, J. Lefort, M. F. Bureau, D. Scherman and B. B. Vargaftig

Am J Physiol Lung Cell Mol Physiol, January 1, 2004; 286 (1): L98-105.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Airway Inflammation and Responsiveness in Prostaglandin H Synthase-Deficient Mice Exposed to Bacterial Lipopolysaccharide

D. C. Zeldin, C. Wohlford-Lenane, P. Chulada, J. Alyce Bradbury, P. E. Scarborough, V. Roggli, R. Langenbach and D. A. Schwartz

Am. J. Respir. Cell Mol. Biol., October 1, 2001; 25 (4): 457-465.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Medline items on this article's topics can be found at <http://highwire.stanford.edu/lists/artbytopic.dtl> on the following topics:

Agriculture .. Corn
Oncology .. Inflammation
Immunology .. Interleukin-10
Medicine .. Airway Inflammation
Medicine .. Airway
Physiology .. Mice

Updated information and services including high-resolution figures, can be found at:

<http://jap.physiology.org/cgi/content/full/88/1/173>

Additional material and information about *Journal of Applied Physiology* can be found at:

<http://www.the-aps.org/publications/jappl>

This information is current as of November 16, 2006 .

IL-10 reduces grain dust-induced airway inflammation and airway hyperreactivity

TIMOTHY J. QUINN,¹ SOMER TAYLOR,¹ CHRISTINE L. WOHLFORD-LENANE,¹
AND DAVID A. SCHWARTZ^{1,2}

¹*Pulmonary, Critical Care, and Occupational Medicine Division, Department of Internal Medicine, University of Iowa, and* ²*Department of Veterans Affairs Medical Center, Iowa City, Iowa 52242*

Quinn, Timothy J., Somer Taylor, Christine L. Wohlford-Lenane, and David A. Schwartz. IL-10 reduces grain dust-induced airway inflammation and airway hyperreactivity. *J. Appl. Physiol.* 88: 173–179, 2000.—To determine whether interleukin-10 (IL-10) could alter the development of grain dust-induced airway disease, we pretreated mice with either saline or IL-10 intravenously, exposed the mice to an inhalation challenge with corn dust extract (CDE), and measured inflammation and the development of airway hyperreactivity. Pretreatment with IL-10, in comparison to saline, reduced the concentration and percentage of polymorphonuclear cells in the lavage fluid 30 min after the inhalation challenge with CDE ($P < 0.05$). In comparison to saline-treated mice, IL-10 did not significantly alter the degree of airway hyperreactivity 30 min after the exposure to CDE. IL-10-treated mice lavaged 18 h after challenge with CDE also exhibited a lower percentage of polymorphonuclear cells in the lavage fluid ($P < 0.05$) and had significantly less airway hyperreactivity than did mice pretreated with the saline placebo ($P < 0.05$). These findings indicate that exogenous IL-10 is effective in reducing airway inflammation and airway hyperreactivity due to the inhalation of CDE.

interleukin-10; neutrophils; asthma; lipopolysaccharide

OCCUPATIONAL AND ENVIRONMENTAL exposure to grain dust can cause a spectrum of clinical syndromes, including asthma, acute and chronic changes in airway reactivity, bronchitis, and progressive irreversible airflow obstruction (5). Among grain handlers, the prevalence of work shift changes in forced expiratory volume in 1 s ($\geq 10\%$ decline) varies between 3.9 and 11% (5). Grain dust exposure causes seasonal decrements in airflow (26) and an accelerated longitudinal decline in forced expired volume in 1 s (23). Recent studies in Barcelona, Spain, suggest that environmental exposure to grain dust significantly affects the health of the exposed population, resulting in a higher incidence of asthmatic attacks (1). Chronic exposure to grain dust can cause irreversible and progressive airway disease. Epidemiological studies performed in North America (40), the United Kingdom (3), and South Africa (47) demonstrate that workers chronically exposed to grain dust are at increased risk of developing chronic cough,

wheeze, and dyspnea, irrespective of smoking habits. Long-term follow-up studies have shown that grain workers (6), as well as other workers exposed to organic dusts (9, 38), have accelerated development of airflow obstruction.

Several lines of evidence indicate that endotoxin is one of the primary agents in grain dust and other organic dusts that cause airway inflammation and airflow obstruction. First, the concentration of inhaled endotoxin in the bioaerosol is strongly associated with the development of acute decrements in airflow among cotton workers (29), swine-confinement workers (13), and poultry workers (44). The concentration of endotoxin in the bioaerosol is the most important occupational exposure associated with the development (40) and progression (38) of airway disease in agricultural workers. Second, physiologically, inhaled endotoxin (34), grain dust (10), or cotton dust (4) can cause airflow obstruction in naïve or previously unexposed subjects. Naïve, healthy study subjects challenged with dust from animal confinement buildings develop airflow obstruction and an increase in the serum concentration of neutrophils and interleukin (IL)-6, all of which are most strongly associated with the concentration of endotoxin (not dust) in the bioaerosol (48). Finally, previous exposure-response studies from our laboratory have shown that inhaled grain dust and endotoxin produce similar physiological and biological effects in humans (10) and mice (39); the concentration of endotoxin in grain dust plays an important role in the acute biological response to grain dust in humans (25) and mice (39); a competitive antagonist for lipopolysaccharide (LPS; *Rhodobacter spheroides* diposphoryl lipid A) reduces the inflammatory response to inhaled grain dust in mice (24); and genetic or acquired hyporesponsiveness to endotoxin substantially reduces the biological response to grain dust in mice (39). Taken together, these studies indicate that endotoxin is an important cause of grain dust-induced airway disease.

IL-10, which was originally called “cytokine inhibitory factor,” is a 35-kDa homodimer produced by Th2 cells, B cells, monocytes, and keratinocytes (17). The major effect of IL-10 is to attenuate the release of proinflammatory cytokines. Although IL-10 has direct effects on neutrophils (28), T cells (46), and B cells (37), its principal target is the macrophage (46). One of the primary effects of IL-10 is the inhibition of macrophage IL-12 production; by inhibiting the release of IL-12,

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

IL-10 decreases the subsequent secretion of interferon- γ (IFN- γ), and this, in turn, decreases the release of cytokine effect on molecules such as tumor necrosis factor- α (TNF- α) (28, 37, 46). The ability of IL-10 to antagonize IFN- γ is macrophage and monocyte dependent, and IL-10 is not an effective inhibitor of IFN- γ in systems in which exogenous IL-12 is present (46). Thus IL-10 targets non-IL-12-secreting (nonactivated) macrophages and inhibits activation and IL-12 secretion (37). Importantly, after intravenous exposure to IL-10, whole blood stimulated by LPS *ex vivo* released significantly less TNF- α and IL-1 β without affecting the release of IL-1-receptor antagonist (IL-1ra) or soluble TNF- α receptor (7). IL-10 substantially reduces the inflammatory response to intravenous endotoxin that appears to be mediated by downregulating the release of TNF- α , IL-8, and IL-12 (45). We have found that serum concentrations of IL-10 can be significantly increased by treating mice with oligonucleotides containing unmethylated cytosine-guanine dinucleotide motifs (41). These studies, which indicate that IL-10 can suppress LPS-induced inflammation, suggest that IL-10 may be effective in preventing the inflammation and physiological effects of grain dust, an airway disease that is primarily mediated by endotoxin.

Given the anti-inflammatory properties of IL-10 and its specific effects on endotoxin-mediated inflammation, we hypothesized that pretreatment with IL-10 would substantially reduce the airflow obstruction and airway inflammation after inhalation of grain dust, an inflammatory response that appears to be primarily mediated by endotoxin.

Methods

Experimental protocol. This investigation was designed to determine whether IL-10 was effective in reducing the physiological or inflammatory response to inhaled grain dust extract. Thus mice were treated with either IL-10 (50 μ g/kg) or saline intravenously 2 h before a 4-h challenge with corn dust extract (CDE). A previous study has shown that IL-10 is effective in protecting mice against staphylococcal enterotoxin B-induced lethal shock if it is given up to 3 h before the challenge (2). Lung physiology and whole lung lavage were performed at 30 min and 18 h after the inhalation challenge with CDE. These time points were chosen because previous studies from our laboratory (12) indicate that lung inflammation begins to decline 24 h after inhalation of CDE. Six mice for each condition were used on the basis of sample-size calculations that included desired power of 80% and that used estimates of lung inflammation from previous work (12).

Animals. C57BL/6 male mice (Jackson Laboratories, Bar Harbor, ME) were obtained at 6 wk of age and used within 2 wk. All animal care and housing requirements set forth by the National Institutes of Health Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources were followed, and animal protocols have been reviewed and approved by the University of Iowa Animal Care and Use Committee. Mice were maintained in wood-chip bedding (Northeastern Products, Warrensburg, NY), with food (Formulab Chow 5008, PMI, Richmond, IN) and water supplied *ad libitum*.

Chemicals. Mice were pretreated with commercially available recombinant murine IL-10 (catalog no. 417-ML-025,

R&D Systems, Minneapolis, MN). Corn dust used in this study was obtained from the air filtration system at an eastern Iowa grain-handling facility. CDE was prepared by mixing 3.0 g of dust in 30 ml (0.1% solution) of Hanks' balanced salt solution purchased from the University of Iowa Tissue Culture Facility. This mixture was vortexed for 2 min and shaken for 1 h at 4°C. Next, the mixture was centrifuged at 800 *g* for 20 min, and the supernatant solution was collected, resulting in CDE. This solution was filter sterilized through a 0.22- μ m filter (Durapore polyvinylidene difluoride filter unit, Millipore, Bedford, MA) and then stored at -70°C before use.

Endotoxin assay. The endotoxin concentrations of the CDE aerosols were assayed by using the chromogenic *Limulus* amoebocyte lysate assay (QCL-1000, Whittaker Bioproducts, Walkersville, MD) with sterile, pyrogen-free labware and a temperature-controlled microplate block and microplate reader (405 nm). The solution was serially diluted in pyrogen-free water (pfw) and assayed. The airborne concentration of endotoxin was assessed by sampling 0.30 m³ of air drawn from the exposure chamber through 47-mm binder-free glass microfiber filters (EPM-2000, Whatman International, Maidstone, UK) held within a 47-mm stainless steel in-line air-sampling filter holder (Gelman Sciences, Ann Arbor, MI). Air-sampling filters were extracted with 30 ml of pfw at room temperature with gentle shaking for 1 h. The solutions were then serially diluted with pfw and assayed for endotoxin. Four air samples were assayed for each exposure at evenly spaced time intervals during each 4-h exposure.

Exposure protocol and monitoring equipment. CDE aerosols were generated into a glass 20-liter exposure chamber by using a Collison nebulizer (BGI, Waltham, MA). High-efficiency particulate-filtered air was supplied to the nebulizer at a flow rate of 17 l/min, and the chamber atmosphere was exchanged at 1 change/min. Endotoxin concentrations in the aerosol were determined by sampling the total chamber outflow. The concentration of endotoxin was 4.2 μ g/m³ in the CDE aerosol used for the mice tested 30 min after exposure and 5.3 μ g/m³ in the CDE aerosols for the mice tested 18 h after exposure.

Lung lavage. Lung lavage was performed 30 min after the inhalation challenge and 18 h after the challenge to assess whether administration of IL-10 altered the degree of lower airway inflammation after inhalation of CDE. Mice were euthanized and underwent *in situ* lavage by using PE-90 tubing inserted into the exposed trachea. The lungs were lavaged with 6.0 ml of sterile pyrogen-free saline. After lavage, the lungs were isolated and frozen in liquid nitrogen and stored at -70°C.

Treatment of bronchoalveolar lavage fluid. Lavage samples were centrifuged for 5 min at 200 *g*, and the supernatant was decanted and frozen at -70°C for subsequent use. Cell concentrations were determined by resuspending the residual pellet in Hanks' balanced salt solution (without Ca²⁺ or Mg²⁺) and counting on a hemocytometer. Cells were placed on a glass slide by using a cytocentrifuge (Cytospin-2, Shandon Southern, Sewickley, PA) and stained by using a Diff Quick Stain (Harleco, Gibbstown, NY).

Lung physiology. Mice were placed in an 80-ml whole body plethysmograph (Buxco Electronics, Troy, NY) ventilated by bias airflow at 0.2 l/min. The breathing patterns and pulmonary function of each individual mouse were monitored over time, and direct measurement was made of respiratory rate, pressure changes within the plethysmograph, and "box flow," which is the difference between the animal's nasal airflow and the flow induced by thoracic movement; this difference varies in the presence of airflow obstruction because of pulmonary

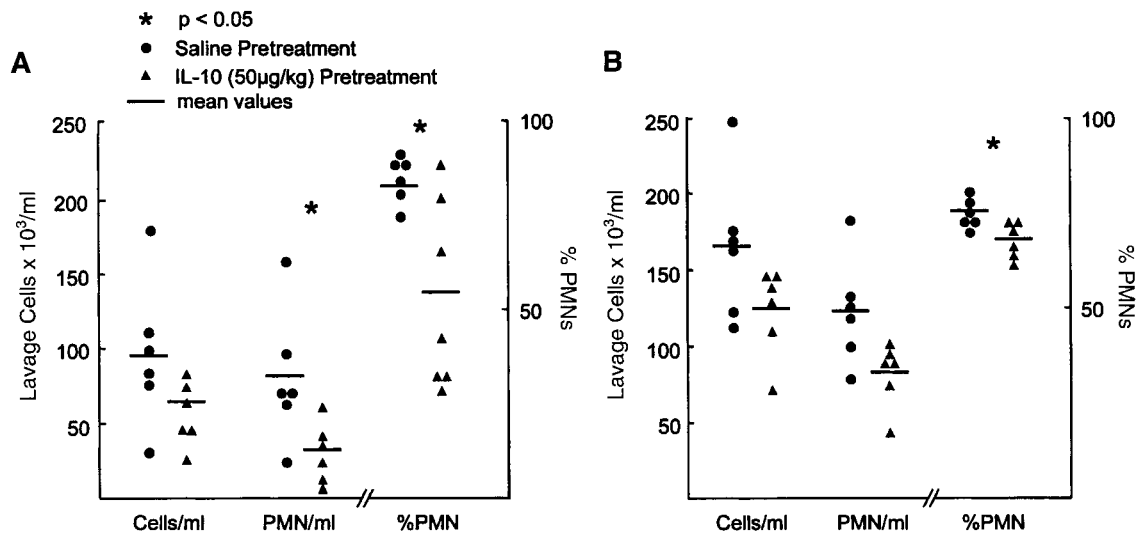


Fig. 1. Lung lavage individual and mean concentration of total cells and polymorphonuclear neutrophils (PMNs) and percentage of PMNs 30 min (A; 4.2 µg endotoxin/m³) and 18 h (B; 5.3 µg endotoxin/m³) after inhalation of corn dust extract in mice pretreated with saline or interleukin (IL)-10. Bars, SE.

compression (due to forced expirations). Airway resistance is expressed as enhanced pause (P_{enh}). $P_{\text{enh}} = (\text{expiratory time} / 40\% \text{ of relaxation time} - 1) \times \text{peak expiratory flow} / \text{peak inspiratory flow} \times 0.67$. The validity of P_{enh} as a measure of bronchoconstriction has been examined (42). P_{enh} was measured at baseline and after stimulation with inhaled methacholine (12.5 and 25 mg/ml) according to a standard protocol (42).

Cytokine analysis of lavage fluid. Lavage fluid was assayed for TNF- α , macrophage inflammatory protein-2 (MIP-2), and IL-1 β . Polyclonal antibodies specific for each recombinant murine cytokine were used as a capture reagent in a standard commercially available sandwich ELISA (R&D Systems). The expression of these specific cytokines and chemokines has been shown to be associated with the acute inflammatory response to inhaled grain dust (12).

Preparation of RNA and multiprobe Rnase protection assay. Total RNA was extracted from lung specimens by using a single-step method, lysing flash-frozen lung in RNA STAT-60 (Tel-Test; Friendswood, TX). The composition of RNA STAT-60 includes phenol and guanidinium thiocyanate in a monophasic solution. The lung parenchyma was homogenized in the RNA STAT-60 by using a polytron homogenizer. Chloroform was then added, and the total RNA was precipitated from the aqueous phase by addition of isopropanol. The total RNA was then washed with ethanol and solubilized in water. After the pellet was dried in a vacuum desiccator, the yield and purity of RNA were quantitated by measuring the ratio of absorbances at 260 and 280 nm. Minigel electrophoresis was used to confirm the integrity of the 28S and 18S rRNA bands. Equivalent amounts of RNA were examined, as judged by the amount of L32, which encodes a ubiquitously expressed ribosome subunit protein (928), in each sample. Commercially available probes were used to detect TNF- α , IL-1 β , and MIP-2 (Pharmingen, San Diego, CA). Densitometry was performed to control for the concentration of RNA in each lane by using Sigma gel (Jandel Scientific Software, San Rafael, CA).

Data analysis. The purpose of this experiment was to compare the inflammatory and physiological response to inhaled CDE between mice pretreated with IL-10 and those pretreated with saline. Inflammation was assessed by using lavage cellularity, lavage fluid cytokine concentrations, and

the relative concentrations of mRNA for specific cytokines in the lung parenchyma. The physiological response to inhaled CDE was evaluated by measuring the airway response to inhaled methacholine. Given the number of mice for each comparison (6 per group), the Mann-Whitney *U* nonparametric statistical test was used for all comparisons (36). A *P* value of <0.05 was considered statistically significant. Six mice for each condition were used on the basis of a sample-size calculation that included a desired power of 80% and anticipated effect of IL-10 of 50% reduction in lung inflammation and that used estimates of lung inflammation from previous work from our laboratory (12).

Results

In a preliminary dosing study, 10 µg/kg IL-10 administered intravenously were ineffective in reducing inflammation due to inhaled LPS. However, 50 µg/kg intravenous IL-10 resulted in a reduced concentration of cells and neutrophils in the lavage fluid of mice challenged with inhaled LPS. On the basis of these

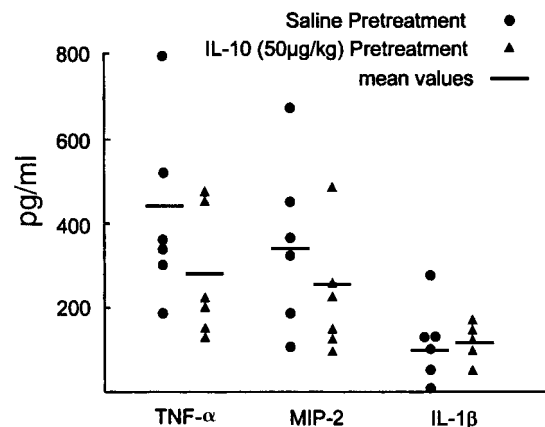


Fig. 2. Lung lavage individual and mean concentration of tumor necrosis factor- α (TNF- α), macrophage inflammatory protein-2 (MIP-2), and IL-1 β 30 min after inhalation of corn dust extract (4.2 µg endotoxin/m³) in mice pretreated with saline or IL-10. Bars, SE.

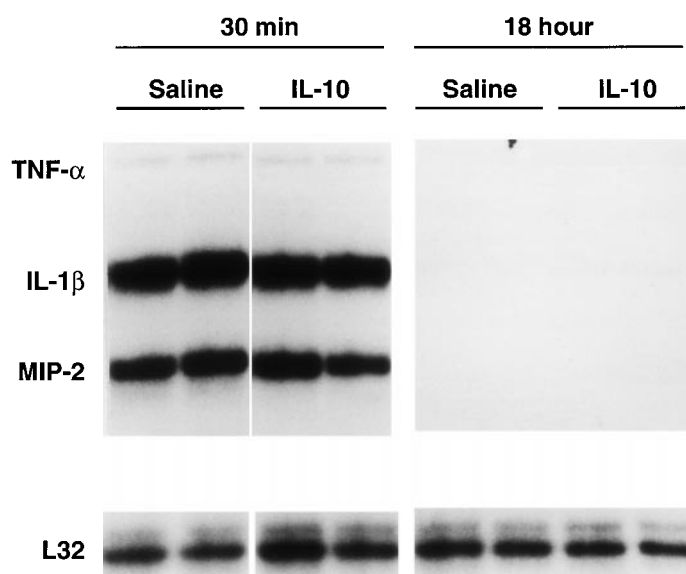


Fig. 3. RNase protection assay of total RNA obtained from lungs after intratracheal instillation of either DNA containing unmethylated cytosine-guanosine dinucleotide (CpG) motifs (bacterial DNA or CpG oligonucleotide) or DNA without unmethylated CpG motifs (eukaryotic DNA, methylated prokaryotic DNA, non-CpG oligonucleotide, or methylated CpG oligonucleotide). Equivalent amounts of RNA were examined in each sample, as judged by the amount of L32, which encodes a ubiquitously expressed ribosome subunit protein.

findings, subsequent experiments with CDE aerosols were performed by using 50 $\mu\text{g}/\text{kg}$.

Pretreatment with exogenous IL-10 reduced the recruitment of inflammatory cells to the lung after inhalation of CDE. Thirty minutes after the exposure to CDE, mice pretreated with IL-10 had a lower concentration of total cells ($P = 0.07$) and neutrophils ($P = 0.02$) and percent neutrophils ($P = 0.03$) in the lung lavage fluid compared with mice pretreated with saline (Fig. 1). This trend was also present 18 h after inhalation of CDE (Fig. 1), although the percent neutrophils was the only statistically significant difference ($P = 0.02$). No differences were observed in the concentration of macrophages, lymphocytes, or eosinophils between mice

treated with IL-10 and saline for either of the time points. The CDE inhalation challenge involved two separate exposures, one for those killed 30 min after the exposure and one for those killed 18 h after the exposure. Thus the absolute increase in total lavage cells observed at 18 h may have been caused by minor differences in the exposure conditions.

Pretreatment with IL-10 did not significantly alter the concentration of TNF- α , IL-1 β , or MIP-2 in the lavage fluid after challenge with CDE (Fig. 2). Thirty minutes after inhalation of CDE, mice pretreated with IL-10 had lower concentrations of TNF- α and MIP-2 in the lavage fluid than did mice pretreated with saline. However, these differences did not prove to be statistically significant. IL-1 β does not appear to be affected by pretreatment with IL-10. The concentration of cytokines in the lavage fluid returned to negligible baseline values 18 h after the exposure, and no differences were observed between IL-10- and saline-treated mice (data not presented). IL-10 did not affect the induction of mRNA for TNF- α , IL-1 β , or MIP-2 in the lung after inhalation of CDE, and 18 h after inhalation of CDE, mRNA for these cytokines was not detectable (Fig. 3).

Pretreatment with IL-10 resulted in less airway hyperreactivity after inhalation of CDE. Figure 4 presents the results of the lung physiology after inhalation of CDE. The P_{enh} was expressed as the percent change from preexposure values (performed 24 h before the inhalation challenge with CDE) at 30 min or 18 h after the inhalation challenge with CDE. Thirty minutes after the inhalation challenge with CDE, mice pretreated with IL-10 had less (albeit not statistically significant) airway hyperreactivity than did mice pretreated with saline. However, consistent and significant differences in airway hyperreactivity were observed 18 h after inhalation challenge with CDE, with IL-10-treated mice demonstrating significantly lower levels of airway hyperreactivity than saline-treated mice. These findings suggest that IL-10 may reduce the degree of airflow obstruction observed after inhalation of grain dust.

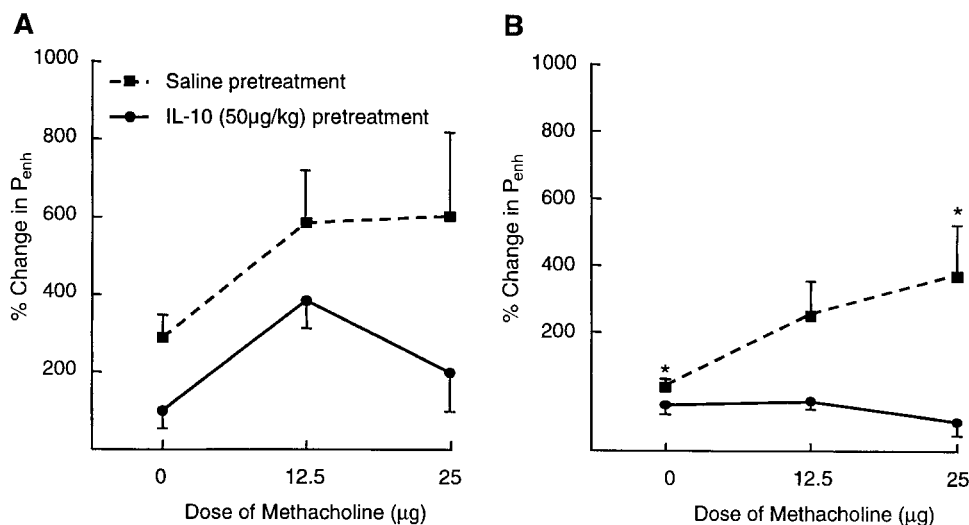


Fig. 4. Methacholine inhalation-percent change from preexposure measures of enhanced pause (P_{enh}) at 30 min (A; 4.2 μg endotoxin/ m^3) and 18 h (B; 5.3 μg endotoxin/ m^3) after inhalation of corn dust extract in mice pretreated with saline or IL-10. Bars, SE.

DISCUSSION

Our results indicate that pretreatment with exogenous IL-10 reduces airway inflammation and airway hyperreactivity after inhalation of CDE. Our results suggest that IL-10 or other anti-inflammatory cytokines may prove to be beneficial in treating grain-dust induced airway disease. Moreover, prevention of the acute biological and physiological response to inhaled grain dust with agents such as IL-10 may reduce the risk of developing chronic grain dust-induced airway disease.

Our findings indicate that IL-10 can decrease the physiological and inflammatory response to inhaled corn dust. Although the specific relationship between acute airway inflammation and the development of chronic airway disease is not fully understood, the present evidence in asthmatic children (31) indicates that recurrent episodes of airway inflammation (not abnormal lung function in the first year of life) result in persistent airway hyperresponsiveness and chronic airway disease. There is increasing evidence among asthmatic individuals that control of the acute inflammatory response substantially improves airflow and chronic airway inflammation (11, 21). Prolonged treatment of newly diagnosed mildly asthmatic (19), chronically stable asthmatic (27), and severely asthmatic individuals (15) with inhaled corticosteroids resulted in significant improvement in airflow. Patients with severe intrinsic asthma treated for 10 yr with inhaled steroids demonstrated reduced airway inflammatory cells and restoration of ciliated epithelia (30). In the aggregate, these findings suggest that control of the acute inflammatory response with agents such as IL-10 may prevent the long-term complications of chronic inhalation of grain dust (3, 6, 40, 47).

Given the importance of endotoxin in mediating organic dust-induced airway disease, one could speculate that several other agents, in addition to IL-10, could effectively prevent the acute response to inhaled grain dust. IL-1ra is an 18- to 22-kDa protein isolated from monocytes (20) and alveolar macrophages (35) that appears to specifically antagonize the IL-1 receptor and has no agonist effects. The production of IL-1ra is triggered by stimuli that differ from those that lead to production of IL-1. For instance, in human volunteers challenged with grain dust, we have found that both protein and mRNA for IL-1 β and IL-1ra are upregulated in the bronchoalveolar space and inflammatory cells (10), suggesting that these cytokines are induced as homeostatic mechanisms. Administration of IL-1ra can block LPS-mediated release of IgE in vitro (43), and infusion of IL-1ra to human volunteers significantly reduced the severity of the response to intravenous LPS (18). Other strategies to control the response to inhaled grain dust could focus on the initial biological response to endotoxin. Such interventions include the use of LPS antagonists (8) and a bactericidal and permeability-increasing protein (16) that binds the lipid A moiety of LPS and prevents stimulation of the CD14 receptor. Because the CD14 receptor is the

primary receptor for endotoxin, interfering with the interaction between the CD14 receptor and endotoxin should significantly reduce the chronic airway response to inhaled grain dust.

Grain dust-induced airway disease serves as an excellent model to investigate the biological features of reversible airway inflammation that are fundamental to the development of chronic asthma. In fact, recent reports have indicated that the concentration of endotoxin in the domestic setting is related to the clinical severity of asthma (33). Moreover, asthmatic individuals develop airflow obstruction at lower concentrations of inhaled endotoxin (32), and inhalation of allergens increases the lung's biological responsiveness to endotoxin (14). Interestingly, inhaled allergens appear to increase the concentration of LPS-binding protein, which allows the lung inflammatory cells to respond to very low concentrations of endotoxin that are commonly present in the airways of uninfected lungs (14). These findings may explain why antigen preparations contaminated with very low levels of endotoxin cause a neutrophilic response in asthmatic subjects (22). Thus agents that prove to be effective in preventing grain dust-induced airway disease may also prove to be effective in asthma. In fact, IL-10 inhibits eosinophil recruitment to the airway in mice sensitized and challenged with ovalbumin (49).

In conclusion, our findings indicate that IL-10 appears to reduce the inflammatory and physiological effects of inhaled CDE. These findings suggest that cytokine regulation is important in controlling the inflammatory response to inhaled grain dust. In addition, our results suggest that IL-10, as well as other anti-inflammatory cytokines, may provide a novel approach to the treatment of grain-dust induced airway disease.

The authors appreciate and acknowledge the support of Dr. Peter Thorne and the Inhalation Toxicology Facility in the Environmental Health Science Research Center.

This study was supported by National Institute of Environmental Health Sciences Grants ES-07498, ES-05605, and ES-09607; National Heart, Lung, and Blood Institute Grant HL-62628; and the Department of Veterans Affairs (Merit Review).

Address for reprint requests and other correspondence: D. A. Schwartz, Pulmonary, Critical Care, and Occupational Medicine Div., Dept. of Internal Medicine, University of Iowa, Iowa City, IA 52242 (E-mail: david-schwartz@uiowa.edu).

Received 28 December 1998; accepted in final form 31 August 1999.

REFERENCES

1. Anto, J. M., J. Sunyer, R. Rodriguez-Roisin, M. Suarez-Cervera, L. Vazquez, and T. Committee. Community outbreaks of asthma associated with inhalation of soybean dust. *N. Engl. J. Med.* 320: 1097-1102, 1989.
2. Bean, A. G. D., R. A. Freiberg, S. Andrade, S. Menon, and A. Zlotnick. Interleukin 10 protects mice against staphylococcal enterotoxin B-induced lethal shock. *Infect. Immun.* 61: 4937-4939, 1993.
3. Blainey, A. D., M. D. Topping, S. Ollier, and R. J. Davies. Allergic respiratory disease in grain workers: the role of storage mites. *J. Allergy Clin. Immunol.* 84: 296-303, 1989.
4. Castellan, R. M., S. A. Olenchock, K. B. Kinsely, and J. L. Hankinson. Inhaled endotoxin and decreased spirometric values. *N. Engl. J. Med.* 317: 605-610, 1987.

5. Chan-Yeung, M., D. A. Enarson, and S. M. Kennedy. Impact of grain dust on respiratory health. *Am. Rev. Respir. Dis.* 145: 476–487, 1992.
6. Chan-Yeung, M., M. Schulzer, L. Maclean, E. Dorken, F. Tan, S. Lam, D. Enarson, and S. Grzybowski. Follow-up study of the grain elevator workers in the Port of Vancouver. *Arch. Environ. Health* 36: 75–81, 1981.
7. Chernoff, A. E., E. V. Granowitz, L. Shapiro, V. E. G. Lonnemann, J. B. Angel, J. S. Kennedy, A. R. Rabson, S. M. Wolff, and C. A. Dinarello. A randomized, controlled trial of IL-10 in humans: inhibition of inflammatory cytokine production and immune responses. *J. Immunol.* 154: 5492–5499, 1995.
8. Christ, W. J., O. Asano, A. L. C. Robidoux, M. Perez, Y. Wang, G. R. Dubuc, W. E. Gavin, L. D. Hawkins, P. D. McGuinness, and M. A. Mullarkey. E5531, a pure endotoxin antagonist of high potency. *Science* 268: 80–83, 1995.
9. Christiani, D. C., T.-T. Ye, D. H. Wegman, E. A. Eisen, H.-L. Dai, and P.-L. Lu. Cotton dust exposure, across-shift drop in FEV₁, and five-year change in lung function. *Am. J. Respir. Crit. Care Med.* 150: 1250–1255, 1994.
10. Clapp, W. D., S. Becker, J. Quay, J. L. Watt, P. S. Thorne, K. L. Frees, X. Zhang, C. R. Lux, and D. A. Schwartz. Grain dust-induced airflow obstruction and inflammation of the lower respiratory tract. *Am. J. Respir. Crit. Care Med.* 150: 611–617, 1994.
11. Cockcroft, D. W. Airway hyperresponsiveness: therapeutic implications. *Ann. Allergy* 59: 405–414, 1987.
12. Deetz, D. C., P. J. Jagielo, T. J. Quinn, P. S. Thorne, S. A. Bleuer, and D. A. Schwartz. The kinetics of grain dust-induced inflammation of the lower respiratory tract. *Am. J. Respir. Crit. Care Med.* 155: 254–259, 1997.
13. Donham, K., P. Haglund, Y. Peterson, R. Rylander, and L. Belin. Environmental and health studies of farm workers in Swedish swine confinement buildings. *Br. J. Ind. Med.* 46: 31–37, 1989.
14. Dubin, W., T. R. Martin, P. Swoveland, D. J. Leturcq, A. M. Moriarty, P. S. Tobias, E. R. Bleecker, S. E. Goldblum, and J. D. Hasday. Asthma and endotoxin: lipopolysaccharide-binding protein and soluble CD14 in bronchoalveolar compartment. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 270: L736–L744, 1996.
15. Dutoit, J., C. M. Salome, and A. J. Woolcock. Inhaled corticosteroids reduce the severity of bronchial hyperresponsiveness in asthma but oral theophylline does not. *Am. Rev. Respir. Dis.* 136: 1174–1178, 1987.
16. Elsbach, P., and J. Weiss. Oxygen-independent antimicrobial systems of phagocytes. In: *Inflammation: Basic Principles and Clinical Correlates*, edited by J. I. Gallin, I. M. Goldstein, and R. Snyderman. New York: Raven, 1992, p. 606–636.
17. Enk, A. H., and S. I. Katz. Identification and induction of keratinocyte-derived IL-10. *J. Immunol.* 149: 92–95, 1992.
18. Granowitz, E. V., R. Porat, W. Mier, S. F. Orencole, M. V. Callahan, J. G. Cannon, E. A. Lynch, K. Ye, D. D. Poutsika, and E. Vannier. Hematologic and immunomodulatory effects of an interleukin-1 receptor antagonist coinfusion during low-dose endotoxemia in healthy humans. *Blood* 82: 2985–2990, 1993.
19. Haahtela, T., M. Jarvinen, T. Kava, D. Kiviranta, S. Koskinen, K. Lehtonen, K. Nikander, T. Persson, O. Selroos, and A. Sovijarvi. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. *N. Engl. J. Med.* 331: 700–705, 1994.
20. Hannum, C. H., C. J. Wilcox, W. P. Arend, F. G. Joslin, D. J. Dripps, P. L. Heimdal, L. G. Armes, A. Sommer, S. P. Eisenberg, and R. C. Thompson. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* 343: 336–340, 1990.
21. Holgate, S. T., R. Beasley, and O. P. Twentymen. The pathogenesis and significance of bronchial hyperresponsiveness in airways disease. *Clin. Sci. (Colch.)* 73: 561–572, 1987.
22. Hunt, L. W., G. J. Gleich, T. Ohnishi, D. Weiler, E. S. Mansfield, H. Kita, and S. Sur. Endotoxin contamination causes neutrophilia after pulmonary allergen challenge. *Am. J. Respir. Crit. Care Med.* 149: 1471–1475, 1994.
23. Huy, T., K. de Schipper, M. Chan-Yeung, and S. M. Kennedy. Grain dust and lung function: dose-response relationships. *Am. Rev. Respir. Dis.* 144: 1314–1321, 1991.
24. Jagielo, P. J., T. J. Quinn, N. Qureshi, and D. A. Schwartz. Grain dust-induced lung inflammation is reduced by *Rhodobacter sphaeroides* disphosphoryl lipid A. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 274: L26–L31, 1998.
25. Jagielo, P. J., P. S. Thorne, J. L. Watt, K. L. Frees, T. J. Quinn, and D. A. Schwartz. Grain dust and endotoxin inhalation produced similar inflammatory responses in normal subjects. *Chest* 110: 263–270, 1996.
26. James, A. L., M. J. Zimmerman, H. Ee, G. Ryan, and A. W. Musk. Exposure to grain dust and changes in lung function. *Br. J. Ind. Med.* 47: 466–472, 1990.
27. Juniper, E. F., P. A. Kline, M. A. Vanzieleghem, E. H. Ramsdale, P. M. O'Byrne, and F. E. Hargreave. Effect of long-term treatment with an inhaled corticosteroid (budesonide) on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics. *Am. Rev. Respir. Dis.* 142: 832–836, 1990.
28. Kasama, T., R. M. Strieter, N. W. Lukacs, M. D. Burdick, and S. L. Kunkel. Regulation of neutrophil-derived chemokine expression by IL-10. *J. Immunol.* 152: 3559–3562, 1994.
29. Kennedy, S. M., D. C. Christiani, E. A. Eisen, D. H. Wegman, I. A. Greaves, S. A. Olenchock, T. T. Ye, and P. L. Lu. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. *Am. Rev. Respir. Dis.* 135: 194–200, 1987.
30. Lundgren, R., M. Soderberg, P. Horstedt, and R. Stenling. Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. *Eur. Respir. J.* 1: 883–889, 1988.
31. Martinez, F. D., A. L. Wright, L. M. Taussig, C. J. Holberg, M. Halonen, and W. J. Morgan. Asthma and wheezing in the first six years of life. *N. Engl. J. Med.* 332: 133–138, 1995.
32. Michel, O., J. Duchateau, and R. Sergysels. Effect of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. *J. Appl. Physiol.* 66: 1059–1064, 1989.
33. Michel, O., R. Ginanni, J. Duchateau, F. Vertongen, B. Le Bon, and R. Sergysels. Domestic endotoxin exposure and clinical severity of asthma. *Clin. Exp. Allergy* 21: 441–448, 1991.
34. Michel, O., R. Ginanni, B. Le Bon, J. Content, J. Duchateau, and R. Sergysels. Inflammatory response to acute inhalation of endotoxin in asthmatic patients. *Am. Rev. Respir. Dis.* 146: 352–357, 1992.
35. Moore, S. A., R. M. Streiter, M. W. Rolfe, T. J. Standiford, M. D. Burdick, and S. L. Kunkel. Expression and regulation of human alveolar macrophage-derived interleukin-1 receptor antagonist. *Am. J. Respir. Cell Mol. Biol.* 6: 569–675, 1992.
36. Rosner, B. *Fundamentals of Biostatistics* (3rd ed.). Boston, MA: PWS-Kent, 1990.
37. Roussel, F., E. Garcia, T. Defrance, C. Peronne, N. Vezzio, D. Hsu, R. Kastelein, K. W. Moore, and J. Bancherau. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc. Natl. Acad. Sci. USA* 89: 1890–1893, 1992.
38. Schwartz, D. A., K. J. Donham, S. A. Olenchock, W. Popen-dorf, S. D. van Fossen, L. F. Burmeister, and J. A. Merchant. Determinants of longitudinal changes in spirometric functions among swine confinement operators and farmers. *Am. J. Respir. Crit. Care Med.* 151: 47–53, 1995.
39. Schwartz, D. A., P. S. Thorne, P. J. Jagielo, G. E. White, S. A. Bleuer, and K. L. Frees. Endotoxin responsiveness and grain dust-induced inflammation in the lower respiratory tract. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 267: L609–L617, 1994.
40. Schwartz, D. A., P. S. Thorne, S. J. Yagla, L. F. Burmeister, S. A. Olenchock, J. L. Watt, and T. J. Quinn. The role of endotoxin in grain dust-induced lung disease. *Am. J. Respir. Crit. Care Med.* 152: 603–608, 1995.
41. Schwartz, D. A., C. L. Wohlford-Lenane, T. J. Quinn, and A. M. Krieg. Bacterial DNA or oligonucleotides containing unmethylated CpG motifs can minimize LPS-induced inflammation in the lower respiratory tract through an IL-12 dependent pathway. *J. Immunol.* 163: 224–231, 1999.
42. Schwarze, J., E. Hammelmann, G. Larsen, and E. W. Gelfand. Whole body plethysmography (WBP) in mice detects

- airway sensitization through changes in lower airways function. *J. Allergy Clin. Immunol.* 97: 293, 1996.
43. **Sim, T. C., J. A. Grant, K. A. Hilsmeier, Y. Fukuda, and R. Alam.** Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am. J. Respir. Crit. Care Med.* 149: 339–344, 1994.
 44. **Thelin, A., O. Tegler, and R. Rylander.** Lung reactions during poultry handling related to dust and bacterial endotoxin levels. *Eur. J. Respir. Dis.* 65: 266–271, 1984.
 45. **Van der Poll, T., P. M. Jansen, W. J. Montegut, C. C. Braxton, S. E. Calvano, S. A. Stackpole, S. R. Smith, S. W. Swanson, C. E. Hack, and S. F. Lowry.** Effects of IL-10 on systemic inflammatory responses during sublethal primate endotoxemia. *J. Immunol.* 158: 1971–1975, 1997.
 46. **Viera, P., R. De Waal Malefyt, M. N. Dang, K. E. Johnson, R. Kastelein, D. F. Fiorentino, J. E. De Vries, M. G. Roncarlo, T. R. Mosmann, and K. W. Moore.** Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRF1. *Proc. Natl. Acad. Sci. USA* 88: 1172–1176, 1991.
 47. **Yach, D., J. Myers, D. Bradshaw, and S. R. Benatar.** A respiratory epidemiologic survey of grain mill workers in Cape Town, South Africa. *Am. Rev. Respir. Dis.* 131: 505–510, 1985.
 48. **Zhiping, W., P. Malmberg, B.-M. Larsson, K. Larsson, L. Larsson, and A. Saraf.** Exposure to bacteria in swine-house dust and acute inflammatory reactions in humans. *Am. J. Respir. Crit. Care Med.* 154: 1261–1266, 1996.
 49. **Zuany-Amorim, C., S. Haile, D. Leduc, C. Dumarey, M. Huerre, B. B. Vargaftig, and M. Pretolani.** Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. *J. Clin. Invest.* 95: 2644–2651, 1995.

